fluorescein dyes recently have found application as insecticides (Heitz, 1982 in Insecticide Mode of Action, J. Coats, ed. Academic Press). Molecular mechanisms of photosensitized lethality and mutagenicity are only partially understood (In Oxygen and Oxy-Radicals in Chemistry and Biology, M.A.J. Rodgers and E.L. Powers, eds. Academic Press, 1981; Cadet, et al., 1983 Israel J. Chem. 23: 420). Glutathione (GSH) and pyridine nucleotide levels and lipid peroxidation were assayed in house flies (Musca domestica) which were photodynamically treated by feeding 1.0 mM rose bengal, followed by illumination and collection of the resulting moribund insects for comparisons with appropriate controls. Depletion of total GSH, total thiol, reduced GSH, and NADPH were observed; no lipid hydroperoxidation was detected. Depletion of total GSH suggests that GSH is oxidized to some product other than GSSG or is irreversibly conjugated. Chemical depletion of GSH with buthionine sulfoximine resulted in no corresponding increase in sensitivity to photodynamic action. Therefore, unless localized depletion is the case, it appears that GSH depletion is merely incident to, rather than the cause of, photodynamic killing of house flies. Consistent with this interpretation, GSH^+ and GSH^- E. coli are equally sensitive to photodynamic inactivation by illuminated rose bengal solutions.

238

CARCINOGEN – INDUCED HOMOLOGOUS RECOMBINATION BETWEEN DUPLICATED CHROMOSOMAL SEQUENCES IN MAMMALIAN CELLS. Yenyun Wang, Veronica M. Maher, J. Justin McCormick; and R. Michael Liskay*, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824; and Departments of Therapeutic Radiology and Human Genetics, Yale University School of Medicine, New Haven, CT 06510.

Mouse L cells lacking thymidine kinase (tk^-) activity were transfected with a recombinant pSV2-neo plasmid containing two different mutated forms of the Herpes tk gene. These Htk mutations represented two different Xho I linker insertions. Transfectants were selected for G418 resistance and the clones screened by Southern analysis for cells containing a single, stably-integrated copy of the plasmid to serve as target cells for studying the frequency of spontaneous and carcinogen-induced homologous intrachromosomal recombination. Recombinants (w.t. Htk^+ gene) are detected by subsequent selection in HAT medium. Each agent studied, i.e., ^{60}Co, UV, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and anti 7,8-diol-9,10-epoxide of benzo[a]pyrene (BPDE), was tested using doses which lowered the cell survival to between 80% and 10% of the untreated control and each determination was made from $\sim 2 \times 10^6$ surviving target cells. The spontaneous frequency per 10^6 viable cells averaged 18 ± 1. UV, MNNG, and BPDE caused a linear, dose-dependent increase in tk^+ recombinants; δ-rays gave no induction. The order of activity of the agents, compared at 37% survival doses, was: MNNG>, BPDE>, UV>>>,δ-rays. At least 90% of the recombination events appeared to represent gene conversion with the tk^+ cells retaining the Htk gene duplication and the neo gene. In 10% of the events, the neo gene was lost and only a single Htk gene (w.t.) remained (single reciprocal exchange). Southern blot analysis showed that each tk^+ recombinant tested contained an Xho I-resistant (w.t.) Htk gene. No evidence of recombination was found in cells containing only a single mutant copy of Htk. (NIH Grants CA21253, GM32741, and a Leukemia Society Scholar Award.)

239

FIBROBLASTS FROM PATIENTS WITH INHERITED PREDISPOSITION TO RETINOBLASTOMA ARE SLIGHTLY MORE SENSITIVE THAN NORMAL CELLS TO THE CYTOTOXIC EFFECTS OF IONIZING RADIATION, BUT NOT TO ITS MUTAGENICITY. Yenyun Wang, William C. Parks*, Jeffrey C. Wigle*, Veronica M. Maher, and J. Justin McCormick, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824.

Retinoblastoma (RB) is a disease characterized by cancer of the retina developing in early childhood. Fibroblasts from bilateral RB patients, an inherited form of the disease, have been shown to be abnormally sensitive to the cytotoxic effects of ionizing radiation. We compared fibroblasts from 6 bilateral RB patients and 3 normal individuals for their sensitivity to the mutagenic effects of radiation (^{60}Co), using resistance to 6-thioguanine (TG) as the genetic marker. There was no significant difference between the two types of cell lines. The slope of the least squares line representing the frequency of TG resistant cells induced in the RB populations as a function of dose was 23 per 10^6 cells per Gray with an intercept of 0.26 Gray; that for the normal cells was 24 per 10^6 cells per Gray with an intercept of 0.3 Gray. We also compared 8 bilateral RB cell lines and 9 age-matched normal cell lines for sensitivity to the cytotoxic effect of ^{60}Co, using survival of colony-forming ability. The cloning efficiency of the unirradiated RB cell lines ranged from 22% to 76% with an average of 52%; that of the normal cell lines from 21% to 89% with an average of 64%. The mean D_0 for the RB cell lines ranged from 0.99 to 1.69 Gray with an average of 1.44 Gray; that of the normal cell lines ranged from 1.42 to 2.24 Gray, with an average of 1.82 Gray. If these slight differences in sensitivity to the killing effect of ^{60}Co reflect a fundamental difference between RB and normal cells, the mechanism responsible does not apply to the mutagenic response of the cells. (Supported by NCI Grants CA32924, CA21253, CA21289, and DOE Contract EV04659.)